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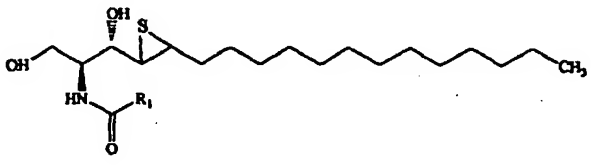
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RADIOSENSITIZER COMPOSITION CONTAINING N-ACETYLPHYTOSPHINGOSINE ANALOGS AND N,N-DIMETHYL-PHYTOSPHINGOSINE ANALOGS AS THE ACTIVE INGREDIENTS



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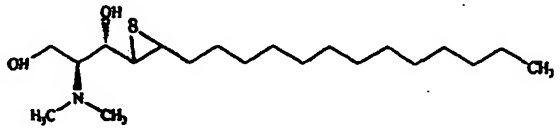

(2)

(57) Abstract: The object of the present invention is to provide a radiosensitizer composition comprises i) 70~97 wt % of N-acetylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 1; and ii) 3~30 wt % of dimethylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 2. Formula 1 wherein, R_1 is C1~C20 alkyl or substituted alkyl S is a double bond; or hydroxyl radical at C4 and hydrogen atom at C5. Formula 2 wherein, S is same as above.

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INTERNATIONAL SEARCH REPORT

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CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, PAJ, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 4025316 A1 (BASF AG) 13 February 1992 (13.02.92) *abstract.*	1-5
A	JP 2001 048721 A (DOOSAN CORP) 20 February 2001 (20.02.01) *paragraph [0024].*	1-5
A	WO 96/18404 A1 (THE BIOMEMBRANE INSTITUTE) 20 June 1996 (20.06.96) *claims.*	1-5
A	WO 2000/59517 A1 (ROGER WILLIAMS HOSPITAL) 12 October 2000 (12.10.00) *claims.*	1-5

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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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„&" document member of the same patent family

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Form PCT/ISA/210 (second sheet) (July 1998)

Radiosensitizer Composition containing N-Acylphytosphingosine Analogs and N,N-Dimethyl-phytosphingosine Analogs as the Active Ingredients.

TECHNICAL FIELD

This invention relates to a radiosensitizer composition containing N-acetylphytosphingosine analogs and dimethylphytosphingosine analogs as the active ingredients. More particularly, this invention relates to a radiosensitizer to be administered before or during the irradiation of radioactive rays for enhancing the cancer therapy of radioactive rays irradiation.

BACKGROUND ART

The cancer has been the first major cause of death and only a 50% of patients can show the complete recovery from the conventional therapy, such as, surgery, radiotherapy and chemotherapy. In Korea, about 35% of cancer patients has been treated with radiotherapy, while about 50% of cancer patients has been treated with radiotherapy in the United States. Therefore, the importance of radiotherapy has been focused according to the increase of the patients treated with radiotherapy. However, there has been some drawbacks of radiotherapy due to the acquirement of radioactive resistance and damage of normal tissue, which causes the decline of therapeutic effect. To overcome the drawbacks of radiotherapy, the radiosensitizer has been researched and developed.

Phytosphingosine is one member of sphingolipid and is a major components of cell membrane. Further, it has various bioactive functions including differentiation, cell cycle arrest and apoptosis. Of course, it also has been induced by not only signals via cell-surface receptors, such as, tumor necrosis factor receptor and Fas ligand but also various stress stimuli, such as, radiation, heat shock, the depletion of growth factors and chemotherapeutic agents.

Ceramide analogs are prepared by the synthesis of free sphingoid bases and free fatty acids in the presence of ceramidase. As sphingoid base, phytosphingosine, sphingosine or sphinganine has been disclosed. Sphingolipids extracted from animals cannot be used any longer, due to the high risk of infection. Further, the chemical synthetic preparation of sphingolipid also has a low yield and a high cost, because different stereotype of isomers are obtained during the chemical synthesis of sphingolipid.

Recently, U.S. Pat. No. 5,958,742 disclosed that sphingolipids are prepared by the biological method using a novel yeast of *Pichia ceferrii*. This biological method for preparing sphingolipids has many merits, since same stereotype of sphingolipids are obtained with a low cost.

On the other hand, U.S. Pat. No. 5,851,782 disclosed the inhibitors of ceramidase. In this disclosure, C2-dihydroceramide is an inhibitor of C2-ceramide, that is to say, the hydration of trans 4-5 double bond of C2-ceramide makes a competitive inhibitor of C2-ceramide.

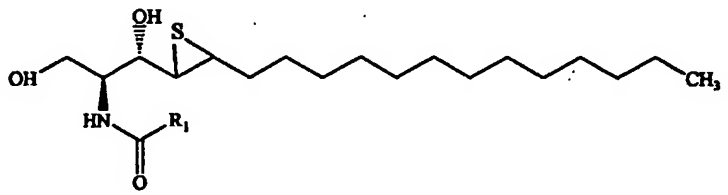
A radiosensitizer composition in the present invention comprises N-acetylphytosphingosine analog of compound of formula 1 and dimethylphytosphingosine analog of compound of formula 2 which is a

metabolic inhibitor of compound of formula 1. Therefore, this radiosensitizer composition leads to sustained ceramide accumulation in cells more efficiently, which sensitizes γ radiation induced cell death.

DISCLOSURE OF INVENTION

The object of the present invention is to provide a radiosensitizer composition comprises i) 70~97 wt% of N-acetylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 1 ; and ii) 3~30 wt% of dimethylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 2.

(Formula 1)

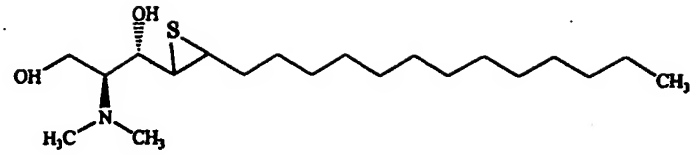


wherein,

R_1 is C1~C20 alkyl or substituted alkyl

S is a double bond ; or hydroxyl radical at C4 and hydrogen atom at C5.

(Formula 2)



wherein,

S is same as above.

Further, among compound of formula 1, R_1 is preferably methyl penthyl or hepthyl, when S is double bond.

The preferred compound of formula 1 is C2 ceramide.

It is preferred that a radiosensitizer composition comprises i) 85~95 wt% of N-acetylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 1 ; and ii) 5~15 wt% of dimethylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 2.

Also, the present invention is to provide the anticancer agent or the cytotoxic agent for radio-resistant and/or chemo-resistant cancer cells comprising the said composition described as above.

BRIEF DESCRIPTION OF DRAWINGS

FIG 1 shows the cancer cell viability by the treatment of N-acetylphytosphingosine.

FIG 2 shows the cancer cell viability by the treatment of dimethylphytosphingosine.

FIG 3 shows the cancer cell viability by the treatment with the combination of N-acetylphytosphingosine and dimethylphytosphingosine according to the increase of irradiating radioactive ray.

FIG 4 shows the change of cell cycle distribution of LLC cell line by the treatment with the combination of N-acetylphytosphingosine and dimethylphytosphingosine after irradiation of radioactive ray.

FIG 5 shows the radiation-induced apoptosis by the treatment with the combination of N-acetylphytosphingosine and dimethylphytosphingosine.

FIG 6a shows the viability of uterine carcinoma cell line with the combination of N-acetylphytosphingosine and dimethylphytosphingosine as radiosensitizer compared to the treatment without radiosensitizer according to the increase of irradiating radioactive ray.

FIG 6b shows the viability of breast cancer cell line with the combination of N-acetylphytosphingosine and dimethylphytosphingosine as radiosensitizer compared to the treatment without radiosensitizer according to the increase of irradiating radioactive ray.

FIG 7 shows the activity change of caspase 3, 8 and 9 by the treatment with the combination of N-acetylphytosphingosine and dimethylphytosphingosine after irradiation of radioactive ray.

FIG 8 shows the increase of Poly-(ADP-ribose)-polymerase (PARP) cleavage by the treatment with the combination of N-acetylphytosphingosine and dimethylphytosphingosine after irradiation of radioactive ray.

FIG 9 shows the change of tumor size after implanting LLC

(Lewis lung carcinoma) cell line to the mouse according to the kinds of treatments.

—■— LLC

----▲---- LLC + Radiation

—▼— LLC/NAPS + DMPS

----◆---- LLC/NAPS + DMPS + Radiation

FIG 10 shows the viability after implanting LLC cell line to the mouse according to the kinds of treatments.

—●— LLC

----○---- LLC + Radiation

—▼— LLC/NAPS + DMPS

----▽---- LLC/NAPS + DMPS + Radiation

FIG 11 shows the radio-resistant cancer cell viability treated with NAPS, DMPS or both treatments.

FIG 12 shows the chemo-resistant cancer cell viability treated with NAPS, DMPS or both treatments.

BEST MODE FOR CARRYING OUT THE INVENTION

In the present invention, it is confirmed that the combination of N-acetylphytosphingosine analog and dimethylphytosphingosine analog as radiosensitizer enables the enhancement of accumulation of ceramide in the human body, and that the radiotherapeutic effect is also enhanced.

According to the radiation treatments of Lewis lung carcinoma cell line, breast cancer cell line and uterine carcinoma cell line, it is also

observed that the viability is rapidly declined on condition that N-acetylphytosphingosine analog and dimethylphytosphingosine analog are used as radiosensitizer. Further, the rapid decline of viability is induced by the synergic effect of apoptosis in combination with the irradiation and the radiosensitizer composition.

N-acetylphytosphingosine analog or dimethylphytosphingosine analog can be used in the form of pharmaceutically acceptable salt or in itself as a drug. The kinds of salts can not be limited if they are pharmaceutically acceptable, and can be illustrated as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, formic acid, acetic acid, tartaric acid, lactic acid, citric acid, fumaric acid, maleic acid, succinic acid, methane sulfonic acid, benzene sulfonic acid, toluene sulfonic acid, naphtalene sulfonic acid salt or its acid addition salt.

The preparation of N-acetylphytosphingosine analog and dimethylphytosphingosine analog composition can obtain any diluent, carrier, additive, stabilizer, emulsifier or auxiliary agent, unless they have any harmful effect. Further, the kinds of formulation of this composition don't have to be limited and can be illustrated as oral formulation, such as, powder, granule, tablet or non-oral formulation, such as, injection.

The weight composition is 70~97wt% of N-acetylphytosphingosine analog and 3~30 wt% dimethylphytosphingosine analog ; preferably 80~95 wt% of N-acetylphytosphingosine analog and 5~20 wt% of dimethylphytosphingosine analog ; the most preferably 85~95 wt% of N-acetylphytosphingosine analog and 5~15 wt% ofdimethylphytosphingo -sine analog. The weight ratio of composition can be varied according to the characteristics of formulation.

The present invention can be explained more concretely by following examples, but the scope of the present invention shall not be limited by following examples.

EXAMPLES

(Example 1)

In this example, N-acetylphytosphingosine analog and dimethylphyto -sphingosine analog is supplied from Doosan corporation.

i) Fixation of N-acetylphytosphingosine concentration

In order to fix the concentration of N-acetylphytosphingosine for measuring the radiosensitizing effect, various concentrations of N-acetylphytosphingosine (1, 3, 5, 8, 10 micro mole) are prepared and treated with Lewis lung carcinoma (LLC) cell lines (2×10^5), and the viability of LLC cells is measured after 24 hours according to the trypan blue dye exclusion method. As shown in FIG 1, the viability of LLC cells is declined in a concentration-dependent manner. In this example, the concentration of N-acetylphytosphingosine is fixed to the concentration showing the 90% of viability of LLC cells, because the purpose of experiment is to confirm the radiosensitizing effect.

ii) Fixation of dimethylphytosphingosine concentration

In order to fix the concentration of dimethylphytosphingosine for measuring the radiosensitizing effect, various concentrations of dimethylphytosphingosine (1, 3, 5, 8, 10 micro mole) are prepared and

treated with Lewis lung carcinoma (LLC) cell lines (2×10^5), and the viability of LLC cell line is measured after 24 hours according to the trypan blue dye exclusion method. As shown in FIG 2, the viability of LLC cells is declined in a concentration-dependent manner. In this example, the concentration of dimethylphytosphingosine is fixed to the concentration showing the 90% of viability of LLC cells, because the purpose of experiment is to confirm the radiosensitizing effect.

(Example 2)

Radiosensitizing effect of cancer cells according to the treatment of N-acetylphytosphingosine and dimethylphytosphingosine

To measure the radiosensitizing effect to the cancer cells of the composition of N-acetylphytosphingosine (NAPS) and dimethylphytosphingosine (DMPS), the 4 kinds of viability of LLC cells are measured before (0 Gy) and after irradiation of radioactive ray (3 Gy) ; i) radioactive ray only, ii) NAPS (5μM) + radioactive ray, iii) DMPS (1μM) + radioactive ray and iv) NAPS (5μM)+ DMPS (1μM) + radioactive ray.

As shown in FIG 3, in case of radioactive ray only, 100% of viability to producing clone at 0 Gy and 76.1% of viability at 3 Gy. There is no significant difference of viability in the treatment of NAPS + radioactive ray or DMPS + radioactive ray compared to the treatment radioactive ray only. However, in the case that radioactive ray is treated with the composition of NAPS + DMPS, the significant decline of viability of LLC cells is shown, that is, 24.5% of viability at 3 Gy.

(Example 3)

Combination effect of NAPS and DMPS treatment on the induction of radiation-induced apoptosis

i) The change of cell cycle distibution after irradiation of radioactive ray

The quantitative FACS analysis is carried out in order to measure change of the LLC cell (4×10^5 cells/ml) cycle distribution before 24 hours of treatment and after treatment to following 4 cases ; i) 5 Gy of radioactive ray only ; ii) 5 micro mole of NAPS + 5 Gy of radioactive ray ; iii) 1 micro mole of DMPS + 5 Gy of radioactive ray ; and iv) 5 micro mole of NAPS + 1 micro mole of DMPS + 5 Gy of radioactive ray. As show in FIG 4, most cells are concentrated in G1 cycle in control group. In the case of treatment with NAPS or DMPS alone, there has been any particular change compared to control group. In the case of group (i), (ii) and (iii) after irradiation, the cell cycles are shifted to G2 cycle. However, most cells are concentrated in sub G1 cycle in group (iv), which proves that apoptosis of LLC cells is increasing in group (iv).

ii) NAPS and DMPS increase the radiation-induced apoptosis by TUNEL assay

To confirm the above results, TUNEL analysis is employed to LLC cells (1×10^5 cell/ml) in the same treatment of i).

The protocol of TUNEL analysis is as follows. After 24 hours of irradiation of radioactive ray, cell lines of above cases are centrifuged and washed. Then, the cells are fixed at the slide using cytospin. The fixed cells are reacted with terminal deoxynucleotidyl transferase

(TdT) and then reacted with horse-radish peroxidase (HRP). After coloring with DAB, the apoptosis of cells is observed with microscope. Apoptosis induced cells are dyed in brown color, whereas viable cells are dyed in blue color. Finally, the degree of apoptosis is measured after counting the number of cells and converting the percentage.

As shown in FIG 5, about 80.7% of apoptosis is measured to the case of [5 micro mole of NAPS + 1 micro mole of DMPS + 5 Gy of radioactive ray], whereas about 8.3% of apoptosis is measured to the case of irradiation of 5 Gy of radioactive ray only. On the other hand, only 4.4% of apoptosis is measured to the case of [5 micro mole of NAPS + 1 micro mole of DMPS] without irradiation of radioactive ray.

(Example 4)

Radiosensitizing effect to the uterine carcinoma cell line and breast cancer cell line

The radiosensitizing effect of NAPS+DMPS is observed to the uterine carcinoma cell line (CT26) and the breast cancer cell line (EMT6) by clonogenic assay. The radiosensitizer enhancement ratio (SER) shows 1.6 to the CT26 and 1.9 to the EMT6. Therefore, it is proved that the composition of NAPS and DMPS also shows the excellent radiosensitizing effect to uterine carcinoma cell line and the breast cancer cell line. The FIG 6 shows the result.

(Example 5)

The change of activation of caspase 3, 8 and 9

To determine the requirement of caspase activities during NAPS and DMPS - induced apoptosis of LLC cells, Western blot analysis is employed to measure the change of activation of caspase 3, 8 and 9. Caspase 3 and 9 are activated in a dose-dependent manner when they are treated with NAPS + DMPS and radioactive ray. However, there has been no significant change of activation in caspase 8, when it is treated with NAPS + DMPS and radioactive ray.

FIG 7 shows the activity change of caspase 3, 8 and 9 by the treatment with the combination of N-acetylphytosphingosine and dimethylphytosphingosine after irradiation of radioactive ray.

The increase of Poly-(ADP-ribose)-polymerase (PARP) cleavage

In addition to the above results, the degree of PARP cleavage is measured using Western blot analysis following 2 cases ; i) changing the degree of radioactive ray (0, 2, 4, 6, 8, 10, 15 Gy) ; and ii) 5 micro mole of NAPS + 1 micro mole of DMPS + changing the degree of radioactive ray (0, 2, 4, 6, 8, 10, 15 Gy).

FIG 8 shows that 86kD cleavage product of PARP is clearly detected when the substrate is treated with NAPS and DMPS with irradiation of 4 Gy of radioactive ray, whereas there was no PARP cleavage in the control LLC cells up to 8 Gy irradiation.

This result suggests that the radiosensitizing activity of NAPS and DMPS are mediated by caspase dependent apoptosis pathway.

(Example 6)

The anticancer activity and radiosensitizing activity in animal model

After implanting the LLC cell line (1×10^6 cells/mouse) to the left leg of C57BL/6 mouse, mice are selected and divided into following 4 groups ; i) control group ; ii) only radioactive ray irradiation group ; iii) NAPS+DMPS treatment group without irradiation of radioactive ray ; and iv) NAPS+DMPS treatment group with irradiation of radioactive ray.

After lapse of 14 days of implantation of the LLC cells, the size of tumor becomes about $1000mm^3$. Then, to the group (iii) and group (iv), 1~15mg/mouse of NAPS and 0.1~1.5mg/mouse of DMPS are administered in abdominal cavity. Also, 20 Gy of ^{60}Co of radioactive ray is irradiated to the group (ii) and group (iv). Then, the growth rate of tumor and the viability of mouse are measured. The growth rate is calculated by (width × length^2)/2 after measuring the size of tumor.

On the other hand, the time required for being $4000mm^3$ of tumor size is measured in above 4 groups. 19 days are required to the control group (i), whereas 27 days are required to the group (iii) which is almost equal to the group (ii). However, 53 days are required to the group (iv), which shows that NAPS + DMPS treatment with irradiation of radioactive ray delays the growth of tumor about 2 times longer than the treatment of radioactive ray only. Table 1 shows the time required for being $4000mm^3$ of tumor size. Also, FIG 9 shows the size of tumor in above 4 groups by the lapse of time.

Further, the viability of group (iii) increases in comparison to that of group (ii). Of course, the viability of group (iv) much increases in

comparison to that of group (iii). The FIG 10 shows the results. Through this experiment, it is confirmed that the composition of NAPS and DMPS has strong radioactive sensitivity in the animal test. As shown in FIG 7, about 40% of viability is shown to the tumor implanted mouse by lapse of more than 50 days.

Table 1.

The comparison of the time required for being 4000mm^3 of tumor size after irradiation of radioactive ray

Group	Required time (day)
Control group (LLC cell)	19
Group (ii) (LLC cell + Radioactive rays (20Gy))	35
Group (iii) (LLC cell + NAPS + DMPS)	27
Group (iv) (LLC cell + NAPS + DMPS + Radioactive rays (20Gy))	53

(Example 7)

Effect of NAPS and DMPS on radioresistant tumor cells

To evaluate the cytotoxic effect of NAPS and DMPS on the radio-resistant tumor cells, various concentrations of radiation (0, 2, 4, 7, 10, 15 Gy), NAPS (5 micro mole) and DMPS (0.5 micro mole) was treated on LLC cells (2X10^5 cell/ml) or LLC-radioresistant cells (2X10^5 cell/ml). The viability of LLC cells is measured after 24 hours by trypan blue dye exclusion method. As shown in FIG 11, the viability of radioresistant LLC cells is higher than LLC cells by increasing the doses

of irradiation. However, there was no difference of the viability between LLC cells and radioresistant LLC cells treated with NAPS and DMPS.

(Example 8)

Effect of NAPS and DMPS on chemoresistant tumor cells

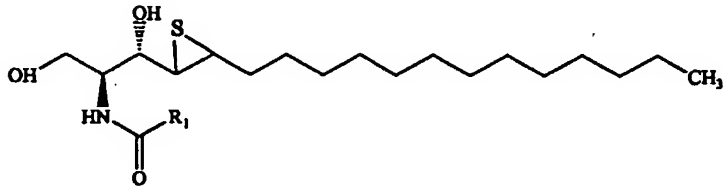
In order to determine the cytotoxic effect of NAPS and DMPS on the chemo-resistant cell line (especially cisplatin-resistant tumor cell), various concentrations of cisplatin (0, 0.5, 1, 5, 30 micro mole), NAPS (0, 0.5, 1, 5, 20, 30 micro mole) and DMPS (0, 0.5, 1, 5, 20, 30 micro mole) was treated on human lung carcinoma A549 cell lines (1 X 10^4 cell/ml) or cisplatin resistant A549 cell lines (1X10^4 cell/ml). The viability of A549 cell line was measured after 24 hours by trypan blue dye exclusion method. As shown in FIG 12, the viability of cisplatin resistant A549 cell line is higher than A549 cell line by treating the cisplatin. However, there was no difference of the viability between A549 cell line and cisplatin resistant A549 cell line treated with NAPS or DMPS and both.

The advantageous effect of the present invention is to provide a radiosensitizer composition to enhance the radioactive therapy without any harmful side effect. Of course, the radiosensitizer composition can be supplied as a medical supporter for treating the cancer patient.

What is claimed is :

1. A radiosensitizer composition comprises i) 70~97 wt% of N-acetylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 1 ; and ii) 3~30 wt% of dimethylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 2.

(Formula 1)

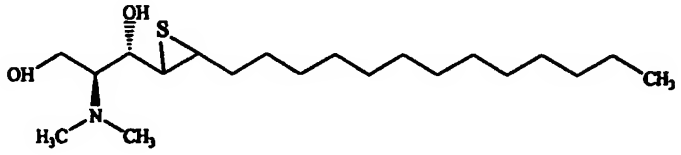


wherein,

R_1 is C1~C20 alkyl or substituted alkyl

S is a double bond ; or hydroxyl radical at C4 and hydrogen atom at C5.

(Formula 2)



wherein,

S is same as above.

2. The radiosensitizer composition according to claim 1, wherein R_1 is substituted or non-substituted methyl, penthyl or hepthyl, when S is

double bond.

3. The radiosensitizer composition according to claim 1, wherein the compound of formula 1 is C2 ceramide.

4. The radiosensitizer composition according to claim 1, wherein a radiosensitizer composition comprises i) 85~95 wt% of N-acetylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 1 ; and ii) 5~15 wt% of dimethylphytosphingosine analog or its pharmaceutically acceptable salt of compound of formula 2.

5. The anticancer agent or the cytotoxic agent for radio-resistant and/or chemo-resistant cancer cells comprising the composition of claim 1.

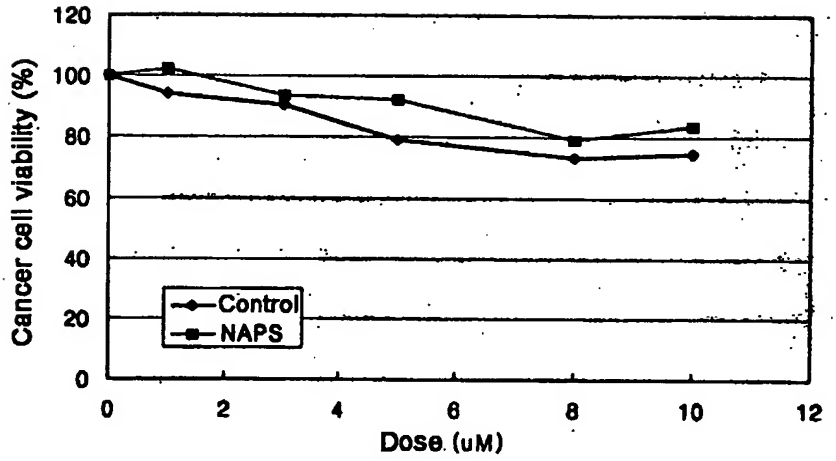

120
100
80
60
40
20
0
Cancer cell viability (%)
Control
NAPS
0
2
4
6
8
10
12
Dose (uM)

FIG 1

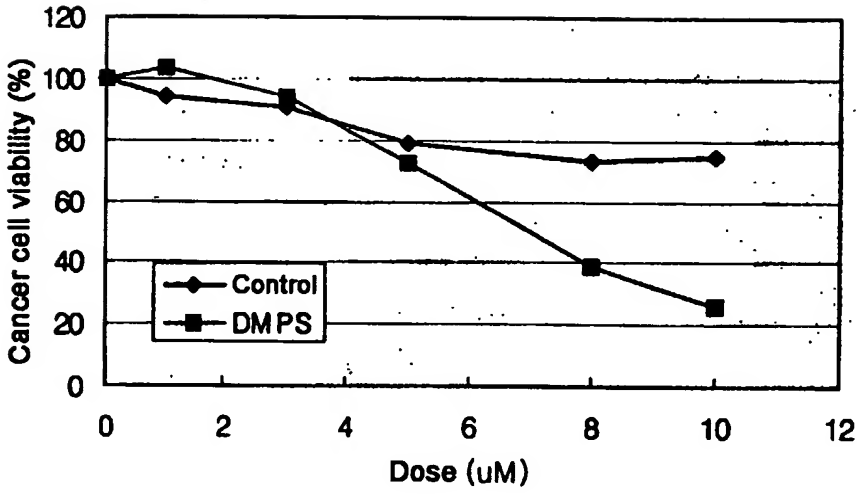

120
100
80
60
40
20
0
Cancer cell viability (%)
Control
DMPS
0
2
4
6
8
10
12
Dose (uM)

FIG 2

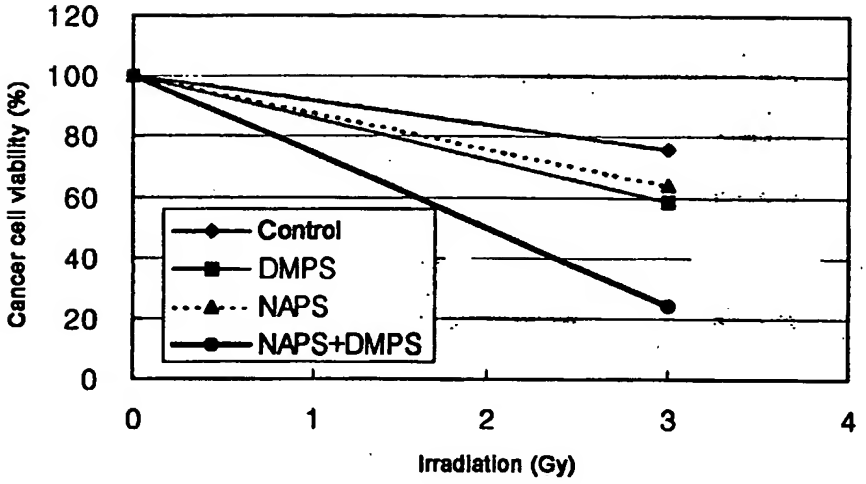

120
100
80
60
40
20
0
Cancer cell viability (%)
Control
DMPS
NAPS
NAPS+DMPS
0
1
2
3
4
Irradiation (Gy)

FIG 3

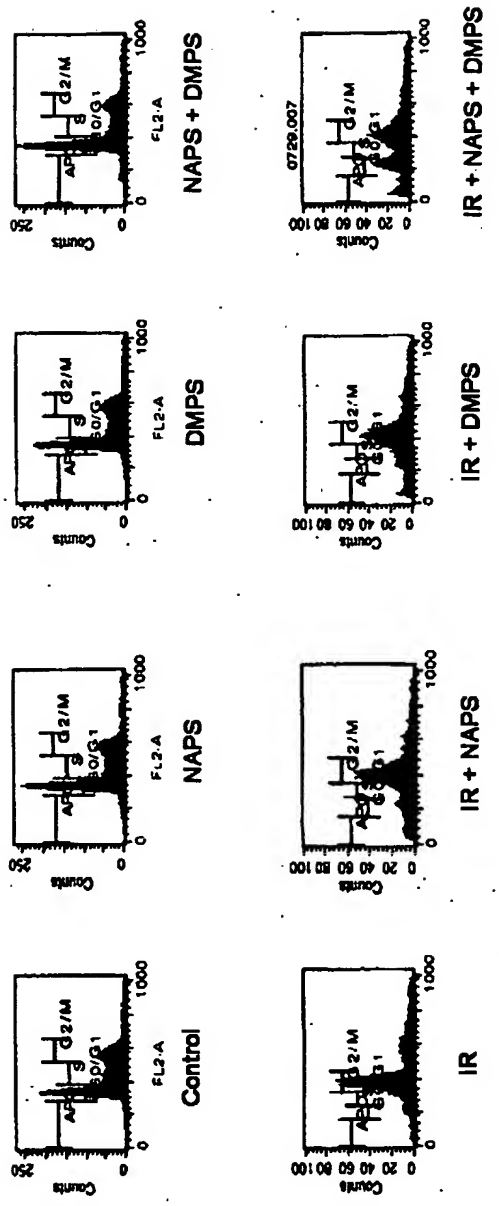

Control
NAPS
DMPS
NAPS + DMPS
IR
IR + NAPS
IR + DMPS
IR + NAPS + DMPS
Counts
FL2-A

FIG 4

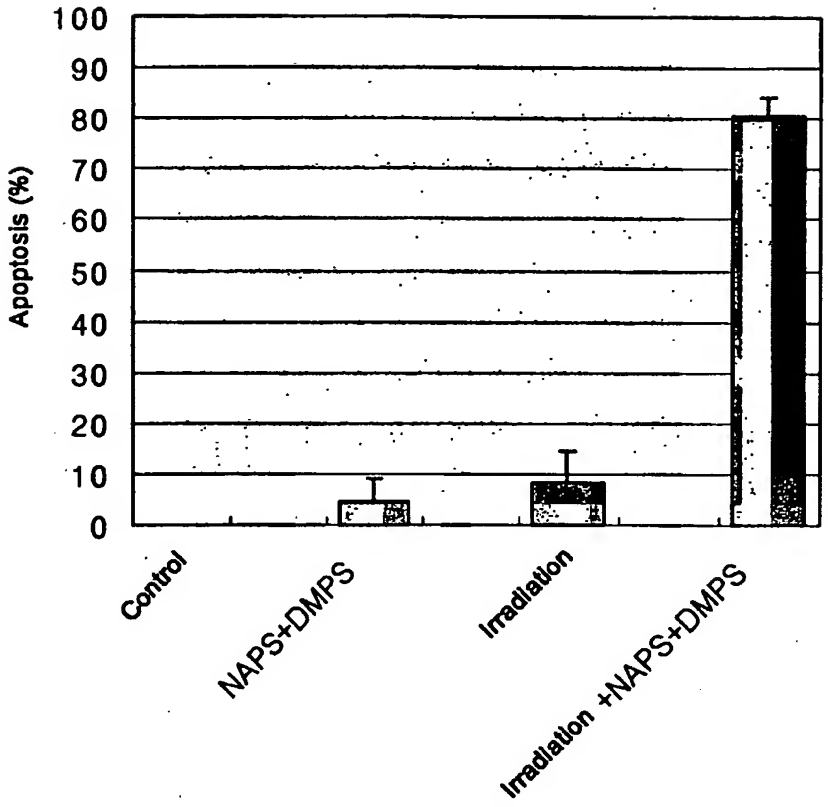

Apoptosis (%)
100
90
80
70
60
50
40
30
20
10
0
Control
NAPS+DMPS
Irradiation
Irradiation +NAPS+DMPS

FIG 5

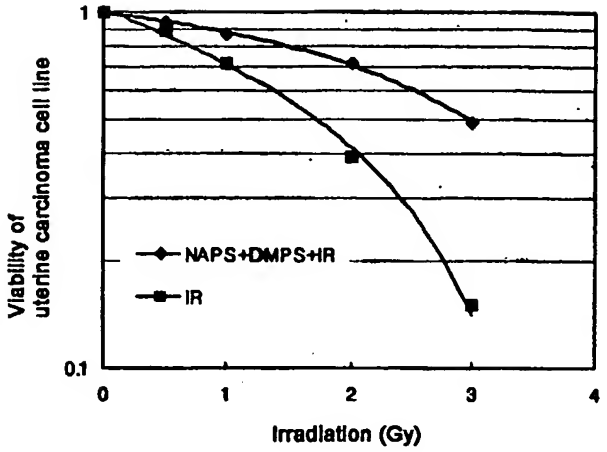

1
0.1
Viability of uterine carcinoma cell line
NAPS+DMPS+IR
IR
0
1
2
3
4
Irradiation (Gy)

FIG 6a

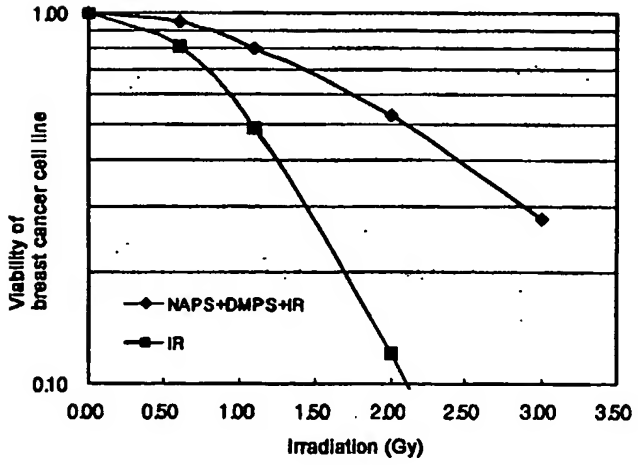

1.00
0.10
Viability of breast cancer cell line
NAPS+DMPS+IR
IR
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
Irradiation (Gy)

FIG 6b

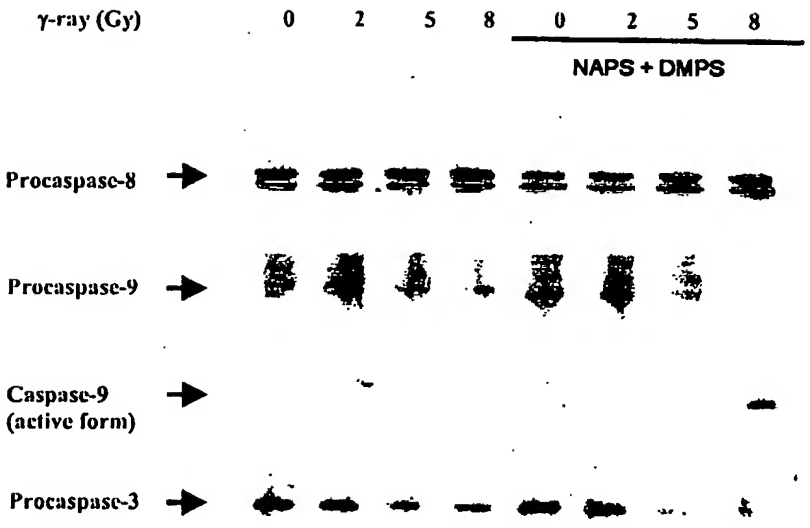

γ-ray (Gy)
0
2
5
8
0
2
5
8
NAPS + DMPS
Procaspase-8
Procaspase-9
Caspase-9
(active form)
Procaspase-3

FIG 7

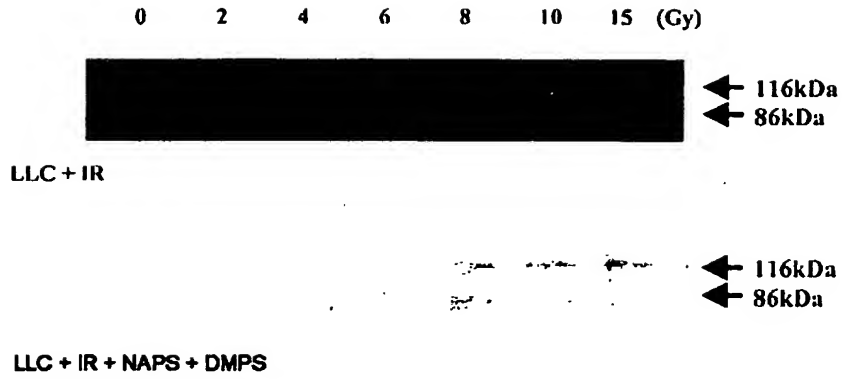

0 2 4 6 8 10 15 (Gy)
116kDa
86kDa
LLC + IR
116kDa
86kDa
LLC + IR + NAPS + DMPS

FIG 8

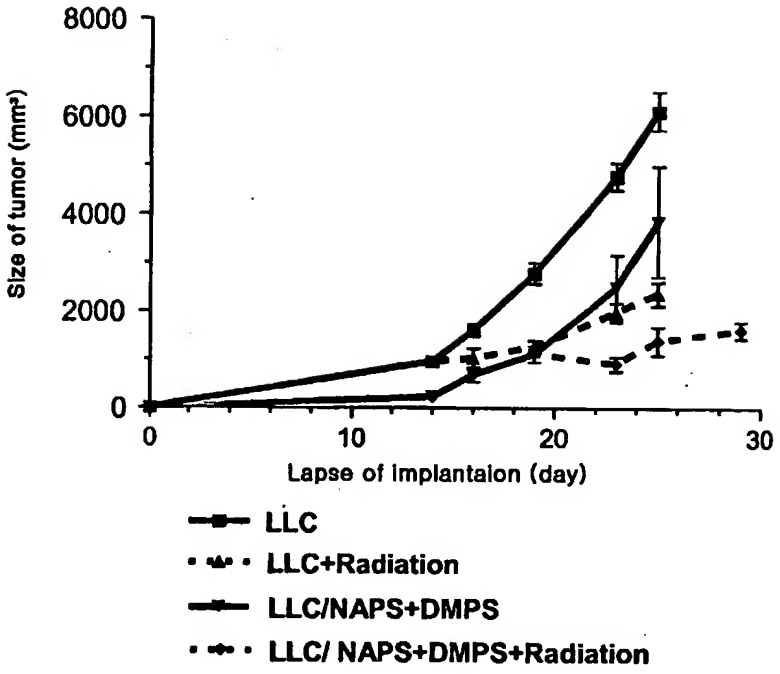

8000
6000
4000
2000
0
Size of tumor (mm³)
0
10
20
30
Lapse of Implantaion (day)
LLC
LLC+Radiation
LLC/NAPS+DMPS
LLC/ NAPS+DMPS+Radiation

FIG 9

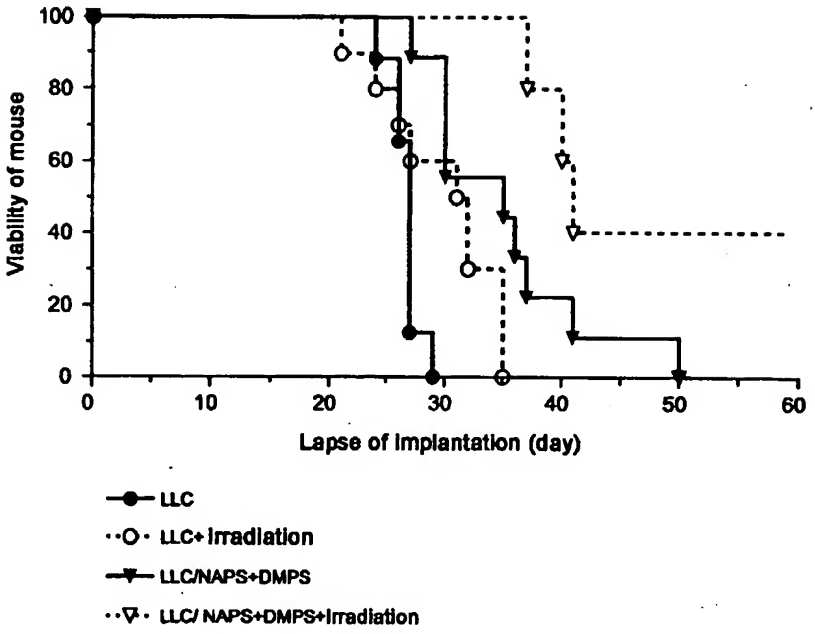

100
80
60
40
20
0
Viability of mouse
0
10
20
30
40
50
60
Lapse of implantation (day)
LLC
LLC+Irradiation
LLC/NAPS+DMPS
LLC/ NAPS+DMPS+Irradiation

FIG 10

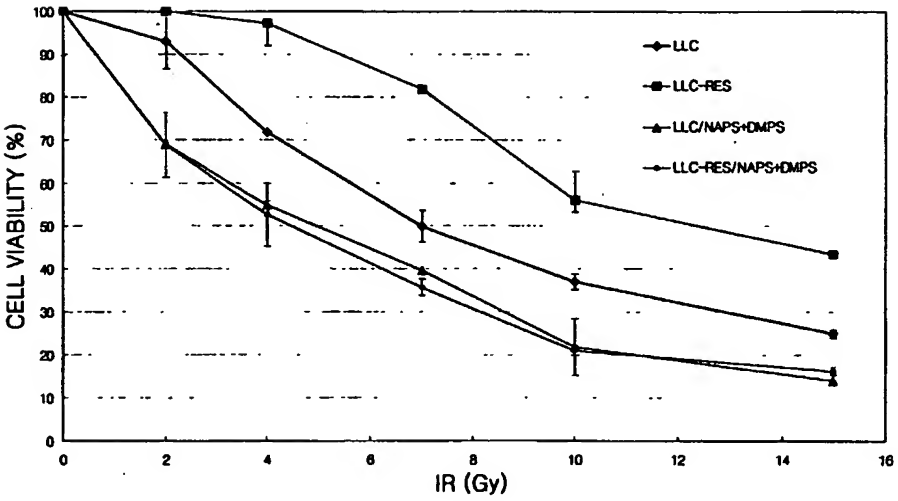

CELL VIABILITY (%)
IR (Gy)
LLC
LLC-RES
LLC/NAPS+DMPS
LLC-RES/NAPS+DMPS
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16

FIG 11

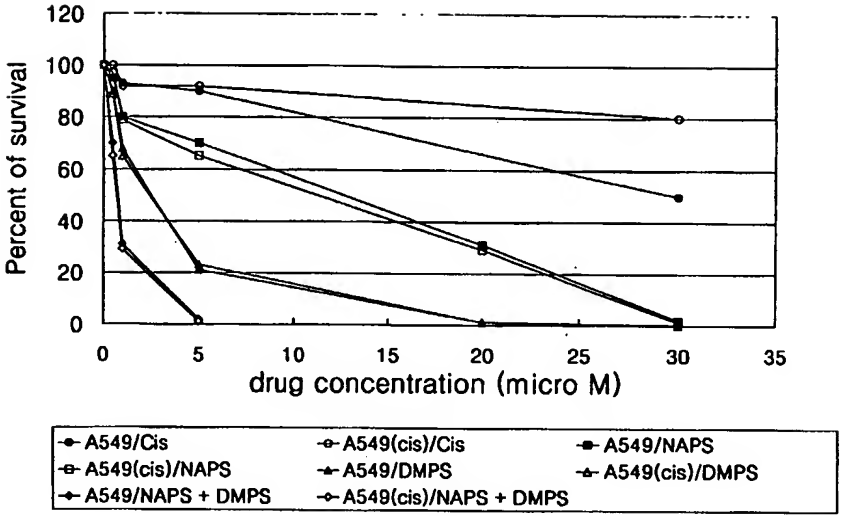

120
100
80
60
40
20
0
Percent of survival
0
5
10
15
20
25
30
35
drug concentration (micro M)
A549/Cis
A549(cis)/Cis
A549/NAPS
A549(cis)/NAPS
A549/DMPS
A549(cis)/DMPS
A549/NAPS + DMPS
A549(cis)/NAPS + DMPS

FIG 12